The Structure of Wortmannin, a Steroidal Fungal Metabolite

By J. MACMILLAN, A. E. VANSTONE,*† and S. K. YEBOAH
(*The Department of Organic Chemistry, The University, Bristol*)

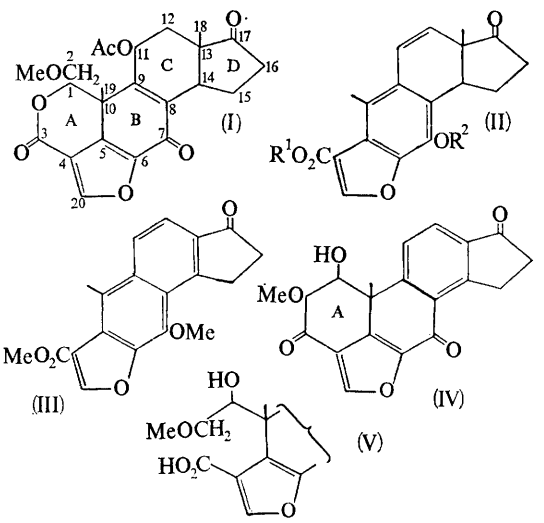
WORTMANNIN was first isolated as a neutral solid, m.p. 240°, from culture filtrates of *Penicillium wortmanni* Klocker by Brian *et al.*[1] who reported that the metabolite showed highly specific antifungal activity and caused a characteristic spiral waving of *Botrytis allii* hyphae. On the chemical and spectroscopic evidence summarised below, we propose structure (I) for wortmannin.

With boiling 2N-hydrochloric acid, wortmannin, $C_{23}H_{24}O_8$,‡ yielded acetic acid (1·0 mol.), methoxyacetaldehyde (0·5 mol.), and two acids, $C_{21}H_{22}O_7$ (0·5 mol.) m.p. 236—238°, and $C_{18}H_{16}O_5$ (0·5 mol.) m.p. 253—256°. Structure (II; $R^1=R^2=H$) was established for the acid, $C_{18}H_{16}O_5$; for brevity, evidence is discussed only for the methyl ester (II; $R^1=Me$, $R^2=H$) m.p. 219—220°. In the n.m.r. spectrum, a sharp one-proton singlet (1·84 τ in $CDCl_3$) was assigned to an α-hydrogen in a furan ring (ν_{C-H} 3150 and 1550 cm.$^{-1}$); this low-field proton was shown to be deshielded by a β-methoxycarbonyl group ($\nu_{C=O}$ 1715 cm.$^{-1}$) by $LiAlH_4$ reduction to a triol, m.p. 209—210·5° in which the low-field proton had moved upfield to τ 2·59. A fully substituted aromatic ring was indicated by the infrared spectrum ($\nu_{C=C}$ 1620 and 1580 cm.$^{-1}$) and by the absence of aromatic proton signals in the n.m.r. spectrum; an aryl methyl group was inferred from the presence of a three-proton singlet (τ 7·3—7·5) in the n.m.r. spectrum of the methyl ester (II; $R^1=Me$, $R^2=H$) and its reduction products and a phenolic hydroxyl group was characterised by the formation of a methyl ether, m.p. 153—154°, and of a monoacetate (1765 cm.$^{-1}$) m.p. 236—237·5°. An AB-system (τ 3·26 and 3·62; J 10 c./sec.) in the n.m.r. spectrum of the methyl ester (II; $R^1=Me$, $R^2=H$) was absent in the dihydro-derivative, m.p. 196—198°, and was consistent with the presence of a *cis*-olefinic double bond, with no allylic hydrogen atoms, and in a six-membered ring. The steroidal five-membered ring was diagnosed by intense peaks in the mass spectra of the methyl ether (II; $R^1=R^2=Me$) at $(M-56)^+$ and $(M-57)^+$ and of its di-deuteriation product at $(M-58)^+$ and $(M-59)^+$. The ring D structure was further supported by reduction of the saturated carbonyl group ($\nu_{C=O}$ 1730 cm.$^{-1}$) with $NaBH_4$ to an alcohol, m.p. 223—225°, whose n.m.r. spectrum in C_5D_5N showed a new one-proton triplet at τ 5·54 (J 7 c./sec.) and a tertiary methyl signal deshielded by τ 0·36 compared to the tertiary methyl signal at τ 9·15 in the original ketone (II; $R^1=Me$, $R^2=H$) in the same solvent.

Orientation of these deduced structural features as in (II) was established by: (i) comparison of the u.v. spectra of the methyl ester (II; $R^1=Me$, $R^2=H$) and of its dihydro-derivative which showed that the double bond was conjugated with the benzene ring; (ii) the upfield shift of the n.m.r. resonance of the aromatic methyl group by

† Present address Biorex Laboratoies Ltd., Research Division, Apsley House, 198 City Road, London, E.C.1.
‡ The molecular formulae of all compounds reported were obtained by high resolution mass spectra on pure crystalline solids.

$LiAlH_4$ reduction of the carboxymethyl group, or by hydrogenation of the double bond, showing the close proximity of these three functions; and (iii) by the small upfield shift in the n.m.r. resonance of the tertiary methyl protons on hydrogenation of the double bond. Structure (II) for the acid, $C_{18}H_{16}O_5$, was finally confirmed by dehydrogenation of the methyl ester methyl ether (II; $R^1=R^2=Me$) with 20% palladium on charcoal to the methyl ester methyl ether (III) of the oxidation product[2] of the mould metabolite, viridin[3] (IV).



From these results, structure (I) can readily be deduced for wortmannin, methoxyacetaldehyde and the acid (II; $R^1=R^2=H$) arising by an acid catalysed retro-aldol reaction after hydrolysis of the lactone and loss of acetic acid. Structure (I) is also supported by spectroscopic data. The i.r. spectrum shows absorption due to the five-ring ketone (1750 $cm.^{-1}$), lactone (1732 $cm.^{-1}$), cyclohexadienone (1684 and 1656 $cm.^{-1}$) and furan ring (3125 and 1540 $cm.^{-1}$). *Inter alia* the n.m.r. spectrum (τ in $CDCl_3$) contains: (i) four methyl singlets of aliphatic methoxyl (6·8), acetoxyl (7·9), and two tertiary methyls (8·28 and 9·02), (ii) an ABX system of the 1-, 2- and 2′-protons; (iii) an AMXY system respectively assigned to the 11-, 14-, 12- and 12′-protons; and (iv) a one-proton singlet at τ 1·78 of the 20-proton. The most intense peaks in the mass spectrum correspond to the separate or successive loss from the molecular ion of 74 (cleavage of ring A at the C(1)–C(10) and C(3)–oxygen bonds), 60 (acetic acid), 57 (ring D cleavage) and 28 [CO from C(3) or C(7)] mass units.

Wortmannin (I) is, therefore, a modified 4-methyl-steroid related to viridin (IV) and ring A of the former may be envisaged as arising from ring A of the latter by fission to (V), then lactonisation.

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[1] P. W. Brian, P. J. Curtis, H. G. Hemming, and G. L. F. Norris, *Trans. Brit. Mycol. Soc.*, 1957, **40**, 366.
[2] J. S. Moffatt, *J. Chem. Soc.*, 1966, 725.
[3] J. F. Grove, P. McCloskey, and J. S. Moffatt, *J. Chem. Soc.*, 1966, 743.